Is Nitric Oxide Involved in the Tonic Inhibition of Central Sympathetic Outflow in Humans?

Jim Hansen, Tage N. Jacobsen, Ronald G. Victor

Abstract Recent studies in experimental animals have advanced the concept that neuronal nitric oxide is an important component of the signal transmission pathways that tonically restrain sympathetic vasocconstrictor outflow from the brain stem. To determine whether or not this concept can be extended to the control of sympathetic outflow in humans, we recorded muscle sympathetic nerve activity (microelectrodes, peroneal nerve) in healthy human subjects during intravenous infusion of the nitric oxide synthase inhibitor N^{G}-monomethyl-L-arginine (L-NMMA) (3.6 to 6.7 mg/kg). The major new finding is that during intravenous L-NMMA mean arterial pressure increased (10±2 mm Hg, P<.05), whereas heart rate and sympathetic nerve activity decreased (P<.05) by 10±2 beats per minute and 61±5%, respectively. These reflex decreases were indistinguishable from those produced when blood pressure was increased comparably with phenylephrine, an internal vasconstrictor control. When the L-NMMA-induced increase in blood pressure was attenuated experimentally to minimize baroreflex activation, sympathetic nerve activity and heart rate were unchanged. Furthermore, during infusion of L-arginine (323 to 513 mg/kg IV) to increase nitric oxide synthesis, mean arterial pressure decreased (12±2 mm Hg, P<.05), but heart rate and sympathetic nerve activity increased (P<.05) by 11±2 beats per minute and 98±27%, respectively. Thus, our experiments in humans provide no support for the emerging concept that nitric oxide is involved in the tonic restraint of central sympathetic outflow. (Hypertension. 1994;24:439-444.)

Key Words • sympathetic nerve activity • nitric oxide • L-arginine • L-NMMA

Studies in experimental animals have provided unequivocal evidence that endogenous synthesis of nitric oxide (NO) from L-arginine is of major importance in the tonic regulation of vasomotor tone and blood pressure.1-3 However, much less is known about the importance of NO in the regulation of the human cardiovascular system. In numerous species, pharmacological inhibitors of NO synthase such as N^{G}-monomethyl-L-arginine (L-NMMA) cause a robust increase in blood pressure that at first was attributed solely to inhibition of endothelial NO synthase.4-9 However, the presence of NO synthase in specific brain regions such as the nucleus tractus solitarius and rostral ventrolateral medulla suggested that NO also might be involved in the central neural control of blood pressure.10-12 Based on these neuroanatomic findings, several recent studies have implicated a major neurogenic component in the blood pressure increase produced by inhibition of NO synthase: it is accompanied by increases in heart rate and sympathetic nerve activity (SNA) and is greatly attenuated by ganglionic blockade.13-17

In intact rats, for example, intravenous L-NMMA produces a sustained elevation in blood pressure with a biphasic response in renal SNA: there is an initial transient decrease in SNA caused by baroreflex activation followed by a sustained increase in SNA caused by central neural activation.13 Thus, the increase in SNA was (1) augmented after denervation of sinoaortic baroreceptors and cardiopulmonary vagal afferents, (2) eliminated by cervical spinal cord transection, and (3) duplicated by microinjection of L-NMMA into the nucleus tractus solitarius in rabbits or the rostral ventrolateral medulla in cats and rats.13-16 Taken together, these extensive studies in experimental animals advanced the concept that the neuronal isoform of NO synthase is an important component of the signal transduction pathways that tonically restrain sympathetic vasoconstrictor outflow from the brain stem. A key unanswered question is whether or not this concept can be extended to the control of sympathetic outflow in conscious humans. To address this question, we recorded postganglionic SNA targeted to the skeletal muscle vasculature in healthy human volunteers with microelectrodes inserted into the peroneal nerve during intravenous infusion of L-NMMA. In designing these experiments in humans, we applied the same reasoning as used previously to identify a sympathoexcitatory effect of intravenous L-NMMA in anesthetized rats.13 If intravenous L-NMMA inhibits mainly endothelial NO synthase, the resultant increase in blood pressure would activate baroreflexes producing monophasic decreases in heart rate and SNA. However, if intravenous L-NMMA also inhibits neuronal NO synthase and thereby reduces the tonic restraint on central sympathetic outflow, an increase in blood pressure would be accompanied by a biphasic response in SNA parallel to that described in rats: an initial baroreflex-mediated decrease in SNA followed by a centrally mediated increase in SNA.

Methods The study protocols were approved by the Institutional Review Board at the University of Texas Southwestern Med-
ical Center, and written informed consent was obtained from each subject before the study. Procedures were in accordance with institutional guidelines. We studied 17 healthy volunteer subjects with a mean age of 27 (range, 20 to 45) years.

General Methods
Subjects were studied in the supine position. Heart rate was measured continuously by electrocardiography; arterial pressure was measured by finger photoplethysmography (Finapres, Ohmeda). Respiratory excursions were monitored with a strain-gauge pneumograph, and during the experimental protocols subjects were instructed to avoid performance of aValsalva maneuver or prolonged expiration because these respiratory maneuvers can stimulate SNA.18 All measurements were recorded continuously on an R-71 FM tape recorder (TEAC) and an electrostatic recorder (model ES 1000, Gould).

Recording of Sympathetic Nerve Discharge
Multiunit recordings of postganglionic SNA were obtained with unipolar tungsten microelectrodes inserted selectively into muscle nerve fascicles of the peroneal nerve posterior to the fibular head by microneurography.19 Briefly, the neural signals were amplified (by 20 to 50×10^3), filtered (bandwidth, 70 to 2000 Hz), rectified, and integrated (time constant, 0.1 second) to obtain a mean voltage display of sympathetic activity. A recording of muscle sympathetic activity was considered acceptable when the neurograms revealed spontaneous, pulse-synchronous bursts of neural activity, with a minimum signal-to-noise ratio of 3:1, that increased during phases II and III of the Valsalva maneuver but not during arousal stimuli (loud noise, skin pinch).

Sympathetic bursts were detected by inspection of the filtered and mean voltage neurograms; the interobserver and intraobserver variabilities in identifying bursts are less than 10% and less than 5%, respectively.20 Inadvertent contraction of the leg muscles adjacent to the recording electrode produces electromyographic artifacts that are easily distinguished from sympathetic bursts; neurograms that revealed such artifacts were excluded from analysis. Nerve traffic was expressed as the number of bursts of sympathetic activity per minute and the number of bursts per minute times mean burst amplitude, an index of integrated nerve activity.

Lower Body Negative Pressure (LBNP)
The subject's lower body was enclosed in a negative pressure chamber to the level of the iliac crest. An opening was created on one side of the chamber to allow performance of the microneurographic technique for recording sympathetic activity from the peroneal nerve in the right leg. Once a stable recording of sympathetic activity was obtained, the opening was closed and sealed during the protocol. The pressure inside the LBNP chamber was measured by a Statham transducer (Gould). LBNP at —10 mm Hg was used to cause venous pooling and thus augment the antihypertensive effect of phentolamine.21

Drug Administration
Investigational New Drug (IND) numbers were obtained from the Food and Drug Administration for the intravenous administration of L-NMMA monacetate (Clinafla AG) (IND No. 41,092 to R.G.V.) and L-arginine hydrochloride (R-Gene 10, Kabi Pharmacia) (IND No. 41,091 to R.G.V.). L-NMMA was dissolved in 50 mL isotonic saline and administered as a constant infusion over 15 minutes. The dose ranged from 3.0 to 6.7 mg/kg. L-Arginine was administered as a constant infusion of 30 g L-arginine in 300 mL isotonic saline over 20 minutes, corresponding to a dose of 323 to 513 mg/kg. L-NMMA and L-arginine caused no adverse effects in any of the subjects.

In some experiments, phenolamine mesylate (Regitine, Ciba Pharmaceutical) was infused through a separate intravenous catheter during the period of L-NMMA infusion (250 mg over 10 minutes), with the phentolamine dose being titrated (dose range, 0.18 to 0.23 mg/kg) to offset the increase in blood pressure produced by L-NMMA. In some experiments, phenylephrine hydrochloride (Neo-Synephrine, Winthrop Pharmaceutical) was infused intravenously, with the dose being titrated (0.5 to 2.0 μg/kg per minute) to match the increases in blood pressure produced by L-NMMA.

Specific Protocols
Protocol 1: Responses to Intravenous L-NMMA (n=7)
Blood pressure, heart rate, and muscle SNA were recorded continuously for 10 minutes at baseline and for 15 minutes during and 25 minutes after intravenous infusion of L-NMMA.

Protocol 2: Responses to Intravenous L-NMMA During Concomitant Administration of Phentolamine During LBNP (n=4)
To determine whether an excitatory effect of L-NMMA on SNA might be masked by concomitant activation of inhibitory baroreflexes (attendant to the L-NMMA–induced elevation in blood pressure), we repeated the L-NMMA infusions while using a combination of LBNP and intravenous phenolamine to offset the increase in blood pressure and thus minimize baroreflex activation. Phenolamine was chosen as a vasodilator whose mechanism of action does not involve NO. We determined in preliminary experiments that to effectively offset the blood pressure–raising effect of L-NMMA, we needed to infuse phenolamine during mild simulated orthostatic stress with LBNP at —10 mm Hg. Thus, blood pressure, heart rate, and SNA were recorded continuously for 5 minutes during LBNP at —10 mm Hg alone and for 10 minutes during concomitant application of LBNP at —10 mm Hg plus intravenous infusions of both L-NMMA and phenolamine, the latter being titrated to maintain blood pressure at the baseline level.

Protocol 3: Responses to Intravenous Phenylinephrine (n=6)
Measurements were repeated in six additional subjects, substituting intravenous phénylinephrine, an internal vasoconstrictor control, for L-NMMA. The phenylamphetamine dose was titrated so as to match the increases in blood pressure produced by L-NMMA.

Protocol 4: Effects of Intravenous L-Arginine on Blood Pressure, Heart Rate, and Muscle SNA (n=6)
Measurements were made continuously for at least 10 minutes at rest, during a 20-minute intravenous infusion of L-arginine, and for at least 25 minutes after the infusion.

Five of the seven subjects that had participated in protocol 1 and one additional subject participated in protocol 4 on a separate day. One of the subjects in these two protocols and three additional subjects participated in protocol 2 on a separate day. Protocol 3 was performed on six additional subjects.

Data Analysis
Statistical analysis was performed using repeated-measures ANOVA with Dunnett's post hoc test to detect values that were different from baseline values. An unpaired t test was used to compare group differences. A value of P<.05 was considered significant. Data are expressed as mean±SEM.

Results
During L-NMMA infusion, mean arterial pressure increased progressively to a value that was 10±2 mm Hg above baseline (P<.05), and heart rate and SNA decreased gradually to values that were 10±2 beats per minute (bpm) and 61±5% below baseline, respectively.
MSNA

Blood Pressure (mmHg)

Baseline

10th min

20th min

Recovery

L-Arg

MSNA

Blood Pressure (mmHg)

Baseline

8th min

16th min

Recovery

L-NMMA

Fig 1. Segments of original recordings from two subjects show mean voltage neurograms of muscle sympathetic nerve activity (MSNA) and corresponding tracings of arterial pressure at baseline and at the mid and end points of an intravenous infusion of either L-arginine (L-Arg, top) or N⁰-monomethyl-L-arginine (L-NMMA, bottom) and during recovery after infusion. L-NMMA produced a sustained increase in blood pressure that was accompanied by a sustained decrease in MSNA. After infusion, blood pressure and MSNA returned toward baseline. Conversely, L-arginine produced a sustained decrease in blood pressure with an accompanying sustained increase in MSNA.

(P<.05) (Figs 1 and 2). After completion of the infusion, all parameters returned gradually to baseline over the following 25 minutes. Neither heart rate nor SNA increased above the baseline values at any point during the 40-minute observation period in any subject.

When blood pressure was held constant during L-NMMA infusion, heart rate and SNA were unchanged from baseline (Fig 3).

When intravenous phenylephrine was used to elevate blood pressure to the same extent as with L-NMMA (10±2 mm Hg, phenylephrine versus L-NMMA), heart rate and muscle SNA decreased by 13±2 bpm and 71±4%, respectively, below baseline (P<.05). These reflex decreases were indistinguishable from those produced by L-NMMA (Fig 4).

During L-arginine infusion, blood pressure decreased gradually to a value that was 12±2 mm Hg below baseline, and heart rate and SNA increased gradually to values that were 11±2 bpm and 98±27%, respectively, above baseline (P<.05) (Figs 1 and 2).

Discussion

These data provide the first measurements of SNA during systemic administration of an NO synthesis inhibitor in humans. The major new finding is that during intravenous L-NMMA blood pressure increased, whereas heart rate and SNA decreased significantly. Heart rate and SNA also did not increase when the L-NMMA-induced increase in blood pressure was prevented experimentally to eliminate a potentially confounding influence of baroreflex activation. When blood pressure was raised to the same extent as achieved with L-NMMA using phenylephrine as an internal vasoconstrictor control, decreases in heart rate and muscle SNA were comparable to those seen with L-NMMA. Furthermore, during intravenous L-arginine to increase NO synthesis, blood pressure decreased, and heart rate and SNA increased. Thus, our experiments provide no support for the emerging concept that NO normally is involved in the tonic restraint of central sympathetic outflow.

Previous studies in humans have demonstrated that intra-arterial administration of L-NMMA causes vasoconstriction in the forearm and coronary circulations, suggesting that endothelial NO synthesis normally contributes to the tonic regulation of vasomotor tone. Our finding that systemic blood pressure increased with intravenous L-NMMA in the setting of decreased heart rate and sympathetic vasoconstrictor drive is consistent with this interpretation.

Previous studies in in vivo and ex vivo animal preparations have advanced the novel concept that neuronal, as well as endothelial, NO synthesis contributes to the tonic regulation of vasomotor tone and blood pressure. Several lines of evidence strongly suggest that neuronal NO is a major component of the signal transduction pathway involved in the tonic restraint of central sympathetic outflow. The neuronal isoform of NO synthase was identified (by staining for NADPH diaphorase) in specific regions of rat brains, such as the nucleus tractus solitarius and rostral ventrolateral medulla, that are mainly involved in the neural
Neuronal NO synthase is a calcium-calmodulin–dependent enzyme that is activated, for example, when calcium influx through \(N\)-methyl-D-aspartate receptor–operated channels is stimulated by L-glutamate.\(^{26,27}\) The key inhibitory neurotransmitter in the nucleus tractus solitarius is NO.\(^{28}\) The evidence that NO mediates glutaminergic inhibition of central sympathetic outflow is that microinjection of L-NMMA into the rat nucleus tractus solitarius inhibited the ability of L-glutamate to cause centrally mediated decreases in blood pressure.\(^{26}\) Further evidence that neuronal NO is involved in the tonic central inhibition of sympathetic outflow in rats, rabbits, and cats is that renal SNA and blood pressure increased with microinjection of L-NMMA specifically into the nucleus tractus solitarius or rostral ventrolateral medulla but not with microinjection into the area postrema or caudal ventrolateral medulla.\(^{14-16}\)

In animal experiments, unlike the present experiments in humans, the sympathoexcitatory effect of NO inhibition has been clearly demonstrated during systemic administration of NO synthesis inhibitors. In anesthetized dogs, the increases in blood pressure produced by NO inhibition (with nitro-L-arginine ester) were virtually abolished by ganglionic blockade.\(^{17}\) In anesthetized rats, increases in blood pressure during L-NMMA were accompanied by a biphasic response in renal SNA consisting of an initial transient decrease followed by a sustained increase in SNA.\(^{13}\) Those findings were interpreted to suggest that intravenous L-NMMA causes a centrally mediated increase in SNA that is partially offset by concomitant activation of inhibitory arterial and cardiopulmonary baroreflexes.\(^{13}\)

That interpretation was supported by the additional findings that the L-NMMA–induced increase in renal SNA was augmented by sinoaortic denervation and vagotomy but eliminated by transection of the cervical spinal cord, the latter suggesting that L-NMMA crosses the blood-brain barrier to activate sympathetic outflow centrally.\(^{13}\)

We considered several potential differences between the previous study in anesthetized rats by Sakuma et al\(^{13}\) and the present study in conscious humans that might have caused us to overlook an important sympathoexcitatory effect of intravenous L-NMMA. First, the L-NMMA dose used in our experiments, 16 to 47 \(\mu\)mol/kg, falls well within the dose range used in the rats (10 to 50 \(\mu\)mol/kg). Second, the 40-minute observation period used in our experiments should have been ample to detect a reversal of the initial sympathoinhibitory response to L-NMMA, which in the rats was evident within 5 to 10 minutes. Third, because the
L-NMMA on muscular sympathetic nerve activity in humans.

The sympathetic excitatory effect of L-NMMA in rats is amplified by baroreceptor denervation and vagotomy, we considered the possibility that in our human subjects baroreflex activation might have masked a sympathoexcitatory effect of L-NMMA. However, this possibility is unlikely because we found no evidence of an increase in muscle SNA with L-NMMA when we used a combination of LBNP and phenolamine infusion to offset the increase in blood pressure and thus decrease the stimulus to arterial baroreceptors.

A fourth possibility might be that central neuronal NO rather selectively regulates sympathetic outflow to the kidney without being involved in the regulation of sympathetic outflow to other target tissues such as in the heart or skeletal muscle. In the rats, however, L-NMMA produced increases not only in renal but also in adrenal SNA, suggesting that L-NMMA causes a rather generalized increase in SNA. This interpretation is inconsistent with our present experiments in conscious humans in which we could find no evidence that L-NMMA causes increases in skeletal muscle SNA.

The two most plausible explanations for the different conclusions derived from the rat and human studies relate to potentially confounding effects of anesthesia and species differences. The blood pressure–raising effect of L-NMMA recently has been found to be much greater in anesthetized than in conscious rats. L-NMMA doses comparable to those used in our human subjects produced increases in blood pressure in conscious rats that were comparable (approximately 10 mm Hg) to those seen in conscious humans but approximately four times larger in either chloralose-urethane- or pentobarbital-anesthetized rats. Given that a substantial fraction of this large increase in blood pressure is thought to be sympathetically mediated, the possibility exists that studies in anesthetized animals may have significantly overestimated the importance of neuronal NO in the regulation of blood pressure.

Another likely possibility is that there may be important species differences in the mechanisms of blood pressure regulation by NO. In support of this interpretation, intravenous l-arginine has no effect on blood pressure and heart rate in conscious rats but reproduc-

Fig 3. Bar graphs show response (mean±SEM) to intravenous N0-monomethyl-L-arginine (L-NMMA) alone (left, n=7) and combined with lower body negative pressure (LBP) and intravenous phenolamine (Phent.) (right, n=4). Muscle sympathetic nerve activity (MSNA) is expressed as total activity in percent of control values. BL indicates baseline. L-NMMA alone significantly increased blood pressure but decreased heart rate and MSNA. When blood pressure was held constant during infusion of L-NMMA with LBNP/phenolamine, both heart rate and MSNA were unchanged from control conditions (LBNP alone). The difference in baseline blood pressure in the two panels is a simple group difference.

Fig 4. Bar graphs compare responses to intravenous N0-monomethyl-l-arginine (L-NMMA, n=7) and intravenous phenylephrine (PE, n=6). Muscle sympathetic nerve activity (MSNA) is expressed as total activity. Shown is mean±SEM change from baseline. When mean arterial pressure (MAP) was elevated comparably with phenylephrine or L-NMMA, reflex decreases in MSNA and heart rate (HR) produced by L-NMMA were indistinguishable from those produced by phenylephrine.
ibly decreases blood pressure triggering compensatory reflex increases in heart rate and now SNA in humans. Our data in conscious humans are consistent with studies in conscious rabbits indicating that the increase in blood pressure with NO inhibition was accompanied by a monophasic decrease in heart rate and was unaffected by ganglionic blockade.7

In conclusion, these studies performed on human subjects argue strongly against an important neurogenic component to the increase in blood pressure produced by systemic administration of an inhibitor of NO synthesis. However, the data do not exclude any role for neuronal NO synthesis in the central neural control of blood pressure in patients with pathophysiological conditions such as chronic renal failure or septic shock, which may produce more profound alterations in NO synthesis than those produced by intravenous L-NMMA in healthy volunteers.33,34

Acknowledgments
This research was supported by a grant to Dr Victor from the Baxter Healthcare Corp. Drs Hansen and Jacobsen are recipients of Fogarty International Research Fellowships (NIH-1-F05-TW04949-01 and NIH-1-F05-TW04949-01) and were supported by grants from the Danish Heart Foundation, the Simonsen & Weel Foundation, and the Danish Research Academy. Dr Victor is an Established Investigator of the American Heart Association. We are indebted to Tolgar Bayraktaroglu for technical assistance and Patricia Powell for secretarial assistance.

References
Is nitric oxide involved in the tonic inhibition of central sympathetic outflow in humans?

J Hansen, T N Jacobsen and R G Victor

Hypertension. 1994;24:439-444
doi: 10.1161/01.HYP.24.4.439

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1994 American Heart Association. Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/24/4/439